Original Research Paper

Volume - 7 | Issue - 5 | May - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Microbiology

Evaluation of anti-diarrheal activity in seed extracts of *Pongamia pinnata* (Fabaceae)

Dahikar S.B. Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, India-423603

Bhutada S.A. Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, India-423603

ABSTRACT The purpose of the present study was to investigate scientifically the anti-diarrheal activity of seed extracts of *Pongamia Pinnata* by castor oil-induced diarrhoea model in Wister albino rats. Anti-diarrheal effect was evaluated by castor oil induced diarrhea. Loperamide 1mg/kg was used as standard drugs. Pet ether, ethanol, aqueous extract at doses 30 mg/kg, 250mg/kg, 100mg/kg respectively was investigated for anti-diarrheal activity in term of reduction in the rate of defecation of faces in castor-oil induced diarrhea. In castor oil induced diarrhea, petroleum ether, ethanol and aqueous extract of *Pongamia pinnata* seeds significantly reduce the mean weight of faces when compared to standard drug. Experimental findings showed that ethanol extract of seeds of *Pongamia pinnata* possess significant anti-diarrheal activity as that of pet ether and aqueous extracts. Therefore it may be a potent source of anti-diarrheal drug in future.

KEYWORDS : *Pongamia pinnata,* anti-diarrheal activity, castor oil

INTRODUCTION

Diarrhea has been one of the most important health problems in developing countries (Sharma *et al.,* 2010). Diarrhea is characterized by increased frequency of bowel movement, wet stool and abdominal pain. A majority of diarrheal cases are due to bacterial enteropathogens, diarrhoeagenic *Escherichia coli (Sairam et al., 2003).* In developing countries they are the most common causes of morbidity and mortality. Many government and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 millin per year (Park, 2000).Many synthetic chemicals like diphenoxylate,loperamide and antibiotics are available for the treatment of diarrhea but they have some side effects. The natural drugs are used as antidiarrheal drugs, which are not always free from adverse effects (Hardman and Limberd, 1992). Therefore, the search for safe and more effective agents has an important area of active research. In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhea (Kouitcheu *et al.,* 2006).

World Health Organization (WHO, 2004) has included a programme for the control of diarrhea, which involves the use of traditional herbal medicine. Several plants have been reported to be used in treating and managing diarrheal diseases. The plant Pongamia pinnata (Fabaceae). Pongamia is a medium sized glabrous tree popularly known as Karanja in Hindi, Indian beech tree in English and Ghanerakaranj in Marathi. It is hardy and can survive in temperatures from 5 to 50 °C and altitudes from 0 to 1200m. Pongamia is native to a number of countries including India, Malaysia, Indonesia, Taiwan, Bangladesh, Sri Lanka and Myanmar. *Pongamia pinnata* possesses antiviral activity, antifungal and antibacterial activity, anti inflammatory activity, ulcer protective activity, and action against infectious diarrhea proved in leaf extract of plant (Salud *et al.,* 2007 and Sangwan *et al.,* 2010). Seeds are compressed ovoid or elliptical, been-like, 10-15cm long, dark brown, oily. Seeds contain 27.5% oil and 13.5% mucilage, also traces of an essential and a complex amino acid named glabrin. Karanjin and pongamol is non-glyceride portion of the oil and are major flavones of the seeds. However, antidiarrheal activity of seed extract of *Pongamia pinnata* had not been carried out so far, hence, this leads us to do study.

MATERIALS AND METHODS

Plant Material

Seeds of *Pongamia pinnata* were collected from the local area of Kopargoan, Maharashtra, India, in month of January and February and the Seeds were identified by Mr. P.S.N. Rao, Join Director, Botanical survey of India, Koregaon road, Pune, The herbarium of the plant specimen has been deposited at B.S.I. Pune, the voucher specimen No. being BRD1. Fresh plant material were washed under running tap water, air dried for two week and then homogenized to fine powder and stored in airtight bottles.

Preparation of extract

The powdered seeds of *Pongamia pinnata* were subjected to successive extraction process using soxhlet apparatus. Powder was packed into soxhlet column and extracted with Petroleum ether (60-80^{0}C) for 24 hrs. The same marc was successively extracted with ethanol for 24 hrs. and afterwards with aqueous extract for 24 hrs. The extract was concentrated under reduced pressure. The dried extracts were stored in airtight container.

Experimental animals

Swiss albino rats (150-180 g) of either sex were selected for the experiments. Institutional Animal Ethics Committee approved the experimental protocol. Animals were housed in polypropylene cages. Animal handling was performed according to Good Laboratory Practice (GLP).

Chemicals and Reagents

Loperamide (Standard reference antidiarrhoeal drug), castor oil (Laxative agent) and vehicle (2% v/v Tween 80 in distilled water) were used.

Phytochemical analysis of the extracts

Phytochemical Screening of Plant materials the presence of saponins, tannins, carbohydrates, alkaloids, flavonoids glycosides, steroids, proteins and alkaloids, were detected by simple qualitative methods(Khandelwal, 2011).

Acute toxicity study

Acute toxicity study for the extract of *Pongamia Pinnata* seeds was carried out for determination of LD_{50} in female albino mice (20-30gm) by adopting fixed dose method LD_{50} study done for finding lethal or effective dose.

Castor oil induced diarrhea

Rats of either sex were fasted for 18h and divided into five groups of six animals per group. Castor oil at a dose of 1ml/animal for the induction of diarrhoea.[9](A)Thirty minutes after castor oil administration ,the first group (control group) received vehicle (0.5%

v/vTween 80 in distilled water),second group received referenced drug loperamide (1mg/kg body weight) while third, fourth and fifth groups were given pet ether, ethanol and aqueous extract at doses of 30mg/kg,250mg/kg,100mg/kg body weight respectively by oral route.

Animals of all groups were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the watery fecal material and number of defecation was noted up to 6h in transparent metabolic cages with filter paper at the base. The total weight of faces were recorded within a period of 6h and compared with the control group.

Statistical analysis

The data are represented as mean ± S.E.M., and statistical significance between treatment and control groups was analyzed using of one-way ANOVA where P<0.05 was considered statistically significant.

RESULT AND DISCUSSION

Karanja (*Pongamia pinnata*) an ancient plant described in Vedas, Samhita and in many Nighantu is a very important medicinal plant. Ethnobotanically it is used in many diseases viz. Diabetes, diarrhea, skin diseases as scabies etc. It is described in Susruta Samhita in Prameha chikitsa and also in many nighantus.To promotes the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Nadkarni, 1954).

Pharmacognosy is the study of medicines derived from natural sources. Under this pharmacognosy, pharmacology and phytochemistry are necessary for authentification of crude drug. Research work was carried out to find the phytochemical constituents and its antidiabetic activity. For this dried seeds of Karanja were taken and then grinded to make fine powder of it, later its ethanolic extract was prepared in Soxhlet apparatus. The photochemical investigation (Table 1) of the various solvent extract of *P. pinnata* showed the petroleum ether extract to contain only alkaloids, and steroids in high concentrations.

Table 1. Phytochemical analysis of *Pongamia pinnata* Seeds

Phytochemical constituent	Aqueous	Petroleum ether	Ethanol
Alkaloids	+	+	+
Flavonoids	-	-	+
Carbohydrates	-	-	+
Glycosides	-	-	+
Amino acids	-	-	-
Saponins	-	-	+
Proteins	-	-	-
Steroids	+	+	+
Tannins	-	-	+

+ = presence, – = absence

Castor oil induced diarrhea

The petroleum ether, aqueous and ethanol extracts was found to be effective against castor oil induced diarrhea on experimental rats at various doses of 30, 250, 100 mg/kg body weight has been shown in Table 1. In petroleum ether, ethanol and aqueous extract of *Pongamia pinnata* seeds significantly reduce the mean weight of faces when compared to control group. Experimental results reflect the activity is more pronounced in ethanol extract at dose 250 mg/kg and subsequently in aqueous extract at dose 100mg/kg and in pet ether extract at dose 30 mg/kg.

Table 2. Effect of extracts of Pongamia Pinnata at different dose levels on castor oil induced diarrhea.

Animal group	Treatment	Dose	Route	Mean weight of stools after 6hrs. otreatment (g±S.D)
I	Control (Tween 80)	5ml/kg	oral	2.57± 0.1411
II	Std.(Loperamide)	1mg/kg	oral	0.50±.060
III	Pet ether extract	30mg/kg	oral	0.83±0.1472
IV	Ethanol Extract	250mg/kg	oral	0.61±0.085
V	Aqueous extract	100mg/kg	oral	0.81±0.1030

Diarrhea is most commonly caused by gastrointestinal infections, which kill around 1.8 million people globally each year, mostly children in developing countries (WHO, 2004). The main cause of death from diarrhea is dehydration, which results from loss of electrolytes in diarrheal stools. Leaves of *Pongamia pinnata* (L.) Pierre (synonym, *P. glabra* vent) has been known as a remedy for diarrhea (Brijesh *et al.,* 2006). Shoba and Thomas, 2001 reported on the effectiveness of *P. pinnata* in controlling castor oil induced diarrhea. While data are available on the effect of medicinal plants on intestinal motility and their antibacterial action, there is a paucity of information on their mode of action on various aspects of diarrheal pathogenicity, namely colonization to intestinal epithelial cells and production/action of enterotoxins (Brijesh *et al.,* 2006). Brijesh *et al.,* (2006) evaluated the crude decoction of dried leaves of *Pongamia pinnata* for its antimicrobial (antibacterial, antigiardial and antirotaviral) effect; and its effect on production and action of enterotoxins (cholera toxin, CT; *Escherichia coli* labile toxin, LT; and *E. coli* stable toxin, ST); and adherence of enteropathogenic *E. coli* and invasion of enteroinvasive *E. coli* and *Shigella flexneri* to epithelial cells. The decoction had no antibacterial, antigiardial and antirotaviral activity, but it was found to reduce the production of CT and bacterial invasion to epithelial cells. These results indicated that the crude decoction of *P. pinnata* has selective antidiarrheal action with efficacy against cholera and enteroinvasive bacterial strains causing bloody diarrhea episodes. They attributed the antidiarrheal activity to antimotility, antisecretory and antimicrobial actions of the compound (Brijesh *et al.,* 2006). .

CONCLUSION

This study provides the rational for the use of the seed extracts of *Pongamia pinnata* as an antidiarrheal drug. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrheal activity observed.

REFERENCES

1. Brijesh S., Daswani P.G., Tetali P., Rojatkar S.R., Antia N.H. and Birdi T.J., Studies on Pongamia pinnata (L.) Pierre leaves: understanding the mechanism(s) of action in infectious diarrhea. J Zhejiang Univ Science B, 2006;7(8):665-74.
2. Hardman JG, Limberd LE. The Pharmacological basis of therapeutics. In: Goodman and Gilman's (Eds), 10th edition, MacGraw Hill, New York, 1992; 914- 931.
3. Khandelwal KR. Preliminary photochemical screening, in: Practical Pharmacognosy Techniques and Experiments, Edn. 8, Nirali Publication, Pune, 2011; 49-156.
4. Kouitcheu Mabeku Laure B., Penlap Beng V., Kouam J. ()Evaluation of antidiarrhoeal activity of the stem bark of Cylicodiscus gabunensis (mimosaceae), African Journal of Biotechnology 2006; 5 (11):1062-1066.
5. Nadkarni A.K., 1954. Indian Materia Medica, Vol. 1, 3rd Ed. Popular Book Depot., Bombay, p.46.
6. Park K. Park's Textbook of preventive and social medicine. Jabalpur, India, M/S Banarsidas Bharat Publishes, 2000;172-175.
7. Sairam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Shankar M. Venkataraman Evaluation of antidiarrhoeal activity in seed extracts of Mangifera indica. J Ethnopharmacology. 2003;84:11–15.
8. Salud Perez Gutierrez. Antidiarroeal activity of different plants used in traditional medicine, 2007; 28-131
9. Savita Sangwan, D.V.Rao and R.A. Sharma, A Review on Pongamia Pinnata (L.) Pierre: A Great Versatile Leguminous Plant, Nature and Science 2010;8(11):130-139
10. Sharma P, Vidyasagar G, Sunder Singh, Ghule S.(). Antidiarrhoeal activity of leaf extract of celosia argentea in experimentally induced diarrhoea in rats; Journal of Advanced Pharmaceutical Technology and Research, 2010; 1(1):41-48
11. Shoba G.F. and Thomas M., Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J. Ethnopharmacol 2001; 76(1):73-6.
12. WHO (World Health Organization), 2004. World Health Report. WHO, Geneva; 120-125.